

Activity in the olivo-cerebello-bulbar system of the cat during ibogaline- and oxotremorine-induced tremor

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Harmaline, an alkaloid of *Peganum harmala*, induces a tremor attributable to rhythmic activity generated in the olivo-cerebello-bulbar system^{7,11,14,20}. It was thus interesting to determine if this system was also responsible for the genesis of tremor induced by other drugs. It has been known for decades that the *Tabernanthe iboga* alkaloids have tremorogenic properties^{17,27}, but little attention has been devoted to the central action of these drugs. The oxotremorine-induced tremor has been shown to have a supra-spinal origin¹⁰, possibly at the level of the mesencephalic reticular formation²¹. The effect of ibogaline and oxotremorine on neurons of the olivo-cerebello-bulbar system were studied in this investigation.

Eight cats were anesthetized with a single dose of Brevital (25 mg/kg, i.v.), decerebrated at the intercollicular level and fixed in the dorsal decubitus position in a stereotaxic apparatus. Using a para-pharyngeal approach, the clivus was removed and the ventral surface of the brain stem exposed. Superficial radial and common sciatic nerves were mounted on bipolar stimulating electrodes. Shortly after the administration of one of the tremorogenic drugs, the animals were paralyzed with Flaxedil (20 mg/kg, i.v.) and artificially ventilated. End tidal CO₂ and temperature were maintained between 3.8-4.2% and 36-38 °C, respectively. The arterial pressure was continuously monitored. Extracellular unitary potentials were recorded with steel microelectrodes insulated with Insul-X. Signals were amplified by conventional means, directly filmed from the oscilloscope and simultaneously recorded on magnetic tape for subsequent computer analysis. At the end of the experiments, the animals were perfused with a 10% formalin solution. Recording sites were marked by iron deposits and later verified in histological sections.

Following the administration of ibogaline (2.5 mg/kg, i.v.), recordings were obtained from the inferior olivary complex, cerebellar cortex, fastigial and brain stem reticular nuclei. As is the case with harmaline^{7,20}, episodes of sustained rhythmic activity at 8-12/sec were recorded from the caudal halves of the medial and dorsal accessory olivary nuclei. Within the rostral halves of these nuclei and in the principal olive, the activity was less regular and had a lower mean frequency. Simultaneous recordings obtained from the caudal accessory olives of both sides usually revealed

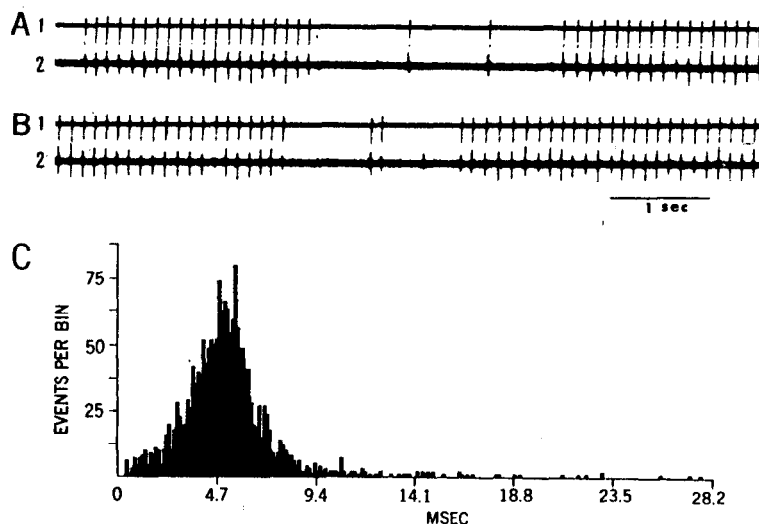


Fig. 1. A and B: simultaneous extracellular recordings from lobule VIII of the cerebellar cortex (trace 1) and contralateral caudal half of the medial accessory olive (trace 2) following administration of ibogaline. C: cross-correlogram of Purkinje and olivary cells shown in A where time zero corresponds to the occurrence of the olivary spikes. The analysis was made on a 13.5 min period of recording (2000 olivary spikes) using a bin width of 125 μ sec.

a high degree of synchronization of discharge. This finding is somewhat surprising since there is no anatomical evidence of inter-olivary connections²².

Purkinje cells of the vermian cerebellar cortex exhibited the same striking characteristics that are observed after the administration of harmaline⁷, namely episodes of sustained rhythmic burst responses at 8–12/sec associated with a suppression of simple spike firing. The harmaline induced bursts have been confirmed by intracellular recordings to be climbing fiber responses²⁰. The activity of two Purkinje cells recorded in lobule VIII (Fig. 1A and B, trace 1) exhibits this behavior. Episodes of regular rhythmic discharge at 8.7/sec occur and there is no simple spike activity.

The simultaneous recordings of olivary activity and of Purkinje cell discharge (Fig. 1A and B) show that each contralateral olivary discharge (trace 2) is associated with a climbing fiber response (trace 1). The extremely precise and constant relationship between these two events is more readily seen in the cross-correlogram (Fig. 1C) between the olivary discharge and the first spike of the climbing fiber response. The mean interval between the olivary discharge and the Purkinje cell activity is 4.8 msec and is compatible with the olivo-cerebellar conduction time⁸. Rhythmic bursts at 8–12/sec were also recorded in the fastigial nucleus and in the bulbar reticular formation after administration of ibogaline.

The high correlation between olivary and Purkinje cell discharges (Fig. 1A and B) does not necessarily imply that the electrodes were recording from two regions directly linked with one another. The demonstration that microiontophoretically applied harmaline within the inferior olive evokes sustained rhythmic bursting of

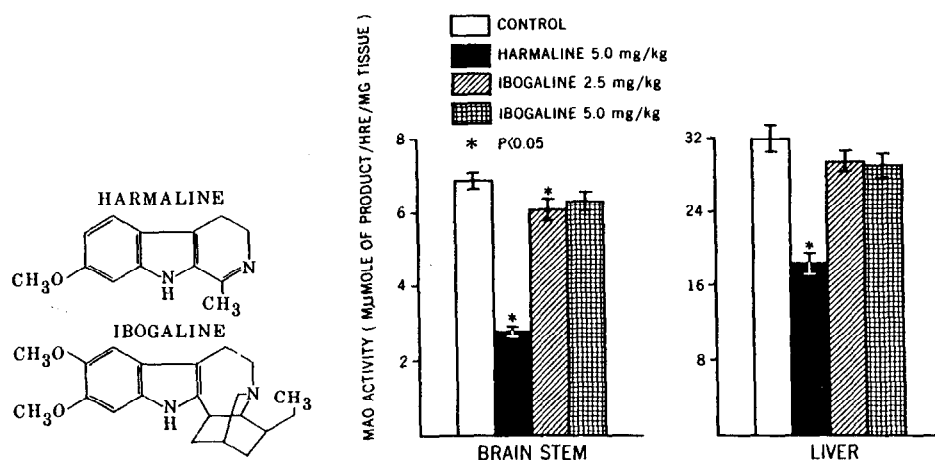


Fig. 2. Structural representation of harmaline and ibogaline.

Fig. 3. Monoamine oxidase (MAO) activity in brain stem and liver of rats injected with harmaline (5 mg/kg, i.v.) or ibogaline (2.5 and 5.0 mg/kg, i.v.). Each column represents the mean of at least 6 animals. The vertical bars are the standard error of the mean. MAO activity was decreased by about 60% in the brain stem and 40% in the liver of rats receiving harmaline. Ibogaline produced a slight decrease of activity (about 10%) only in the brain stem of rats injected with the smaller dose of the drug (2.5 mg/kg).

multi-unit activity¹⁵, suggests that a large number of olivary cells can discharge synchronously in response to the tremorogenic agent. Further support for this hypothesis can be derived from the recent demonstration of strong electrotonic coupling between olivary cells^{19,23}.

In summary, these results demonstrate that ibogaline tremor, like harmaline tremor, is associated with specific activation of the olivo-cerebello-bulbar system. Moreover, we have verified in monkeys that ibogaline, like harmaline, does potentiate the experimental Parkinson-like tremor initiated by ventromedial lesion of the mid-brain (Dumont and Lamarre, unpublished observations). However, the olivo-cerebellar system cannot be responsible for the Parkinson-like tremor since such a tremor persists after total cerebellectomy and subsequent degeneration of inferior olivary cells¹². Abnormal neuronal activity at the thalamo-cortical level appears to be responsible for the Parkinson-like tremor¹³.

The similar effect of ibogaline and harmaline on the olivo-cerebello-bulbar system may be related to the fact that these drugs possess definite structural similarities (Fig. 2). In view of a possible involvement of monoamines in the production of certain kinds of tremor²¹, it was of interest to determine if ibogaline was a monoamine oxidase inhibitor like harmaline²⁵. This was investigated in a series of 26 male Sprague-Dawley rats weighing 230–250 g. Groups of rats were injected i.v. with harmaline 5 mg/kg, or with ibogaline in doses of 2.5 mg/kg or 5.0 mg/kg. The animals were sacrificed 30 min later and MAO activity was measured in brain stem and liver. The results obtained from the experimental groups were compared with a group of control animals injected with physiological saline. The MAO activity was assayed

by measuring the formation of [^{14}C]indoleacetic acid from [^{14}C]tryptamine after an incubation of 20 min at 37 °C as described by Wurtman and Axelrod²⁶. The results obtained are shown in Fig. 3.

With harmaline, MAO activity was decreased by approximately 60% in the brain stem and by 40% in the liver. Ibogaline caused only a slight decrease of 10% ($P < 0.05$) in the brain stem of rats injected with 2.5 mg/kg. This represents the maximum MAO inhibition that could be achieved since this effect was not statistically significant in rats injected with 5 mg/kg of the drug. In the liver, there was no significant decrease in MAO activity with ibogaline at either dose.

These results indicate that ibogaline, contrary to harmaline, produces very little, if any, inhibition of the MAO. This supports the suggestion of Coates and Cox² that monoamine oxidase inhibition is not responsible for the tremorogenic properties of harmine.

Oxotremorine (250 $\mu\text{g/kg}$, i.v.) was given to three cats previously treated with Probanthine (2 mg/kg, i.m.) to prevent the peripheral cholinergic effects of oxotremorine. In each case the microelectrode was stereotactically positioned in the caudal part of the medial accessory olive. The accuracy of the placement was verified by recording typical olivary responses to peripheral nerve stimulation¹.

The animals were paralyzed only after a generalized tremor had appeared following oxotremorine injection. Despite this confirmation of tremorogenesis, no rhythmic activity was ever recorded from any part of the inferior olivary complex, cerebellar cortex or fastigial nucleus. The brain stem reticular formation was not thoroughly investigated, but recordings obtained from the bulbar reticular nuclei (gigantocellularis, magnocellularis and paramedian) also failed to disclose any rhythmic activity.

These results indicate that, contrary to ibogaline and harmaline, oxotremorine tremor is not generated by the olivo-cerebellar system. This is in agreement with previous data. Tasker and Kertesz²⁴ reported that oxotremorine tremor in the rat is not abolished by cerebellectomy. Moreover, it is possible to differentiate harmaline and oxotremorine tremors pharmacologically since only the latter is blocked by anticholinergic drugs^{3,5,9,18}. A difference in origin of these two tremors has already been suggested by Lamarre and Weiss¹⁶, since different spectra of spinal motoneurons seem to be activated by oxotremorine⁴ and harmaline¹⁶.

In conclusion, the results presented in this paper indicate that ibogaline tremor, like harmaline tremor, is generated by specific activation of the climbing fiber afferent system in the cat. This system, however, is not rhythmically activated by oxotremorine. The mesencephalic reticular formation is possibly the site of action of oxotremorine²⁴ while ibogaline presumably acts, like harmaline^{6,15}, directly on neurons of the inferior olivary complex.

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